Evaluation of Effect of Azithromycin on The Heart of Adult Male Albino Rats and The Possible Protective role of VIT.C (Histological and Immune-histochemical Study)

Abeer F. Abd El-Naeem[a] , Azza M.A. Abouelella[b], Asmaa S. Baset[a]*

[a]Department of Human Anatomy & Embryology, Faculty of Medicine, Sohag University, Sohag, Egypt.

[b]Department of Pharmacology, Faculty of Medicine, Sohag University, Sohag, Egypt.

Abstract

Background: Azithromycin is one of the most common drugs used in the protocol of treatment of pneumonia caused by COVID-19. But many researchers approved its toxicity on heart tissue. VIT C is an available and strong antioxidant that has a protective effect against many toxins and drugs. This study aimed to study the cardiotoxic effect of Azithromycin and the possible protective effect of VIT.C against it .

Materials: Thirty adult male rats were used in this study and were divided into 3 groups, the control group with no treatment. Azithromycin group; the rats were treated with Azithromycin (30mg/kg) orally. VIT C group rats were orally treated with the same dose of Azithromycin +VITC (20mg/kg), and drugs were administered for 2 weeks. The results were examined with a light microscope (H & E, Masson, caspase-3, and TNF-α).

Results: Azithromycin exerted significant deleterious effects on heat tissues in the form of distortion of the normal shape, fragmentation of myocardial fibers, and destruction of the cells. Additionally, collagen fibers increased in the azithromycin group, and with immunohistochemistry, the tissues showed a positive reaction to the antigens of caspase-3 and TNF-α. VIT.C ameliorated these detrimental effects.

Conclusion: Azithromycin drug-induced cardiotoxicity should be used in limited cases. The toxic effects of azithromycin on the heart can be potentially reduced by treatment with VIT.C.

Keywords: Azithromycin; VIT C; Antioxidant; Caspase; TNF- α.

DOI: 10.21608/svuijm.2022.155063.1375

***Correspondence**: azza_mallak@yahoo.com

Received: 11 Augst,2022.

Revised: 5 Septembre,2022.

Accepted: 5 Septembre,2022.

Cite this article as: Abeer F. Abd El-Naeem , Azza M.A. Abouelella, Asmaa S. Baset. (2022). Evaluation of Effect of Azithromycin on The Heart of Adult Male Albino Rats and The Possible Protective role of VIT.C (Histological and Immune-histochemical Study). *SVU-International Journal of Medical Sciences*. Vol.5, Issue 2, pp: 518-532.

Introduction

Azithromycin is a widely and effectively used macrolide antibiotic. The macrolides such as erythromycin, clarithromycin, azithromycin, and telithromycin, are the most common antibiotics used to treat infections caused by gram-positive bacteria (**McMullan and Mostaghim, 2015**).

Azithromycin has also additional effects on host-defense reactions and chronic human diseases so it has a special interest in recent years (**Parnham et al., 2014**).

Azithromycin can decrease virus entry into cells, also it can improve immune response against viruses by up-regulating the production of type I and III interferons (especially interferon-β and interferon-λ) and genes involved in virus recognition such as MDA5 and RIG-I. This is why, it is the antibiotic of choice in response to infectious agents potentially against COVID-19 **(Fohner et al., 2017).**

Using azithromycin early in the protocol of treatment significantly improved clinical outcomes as the length of stay or the need for respiratory support during hospitalization **(Martín-Loeches et al., 2013).** While its clinical effects, cardiovascular adverse effects associated with azithromycin have attracted attention such as prolonged Q-T interval, malignant arrhythmia, and sometimes sudden deaths due to ventricular arrhythmia reported as a result of its use (**Ray et al., 2013**). A large retrospective cohort study suggested an increase in cardiovascular deaths in people is related to their treatment with azithromycin compared to other antibiotics (**Ray et al., 2013**).

VIT. C is an essential nutrient that cannot be synthesized by the body and plays an important role in the body's immune-modulating system **(Padhani et al., 2021).** When the free radicals in the human body increase more than antioxidants, this is called oxidative stress **(Mc Gregor and Biesalski,2006; Autifiet al., 2018 ; Li et al., 2018).**

VIT.C contains electrons that protect the body from oxidant damage generated through exposure to toxins so it is considered a strong antioxidant. The 3fiological functions of VIT.C are based on its ability to reduce equivalents for a variety of biochemical reactions. So VIT.C can destroy free radicals and reduce reactive oxygen species and reactive nitrogen species (e.g., superoxide, peroxynitrite, hydroxyl, peroxyl, and nitroxide radicals) **(Arrigoni and De Tullio, 2002; Ergul et al., 2010; Hemilä, 2017**).

Antioxidant effects of VIT. C has been used as a factor in the pathophysiology and histopathology of various health disorders **(Shireen et al., 2018; Budin et al., 2011).** Additionally, VIT.C has a cardioprotective effect as it can ameliorate the altered oxidative stress biomarkers **(Abdel-Daim et al., 2015).** VIT. C also attenuated the caspase-3 expression suggesting anti-inflammatory and antiapoptotic cardioprotective mechanisms against many toxins or drugs-induced cardiotoxicity **(El-Shitany and El-Desoky, 2016).**

VIT.C has also a role in the development and regulation of cancer growth. So it is a potential anti-tumor

factor against cancer cells and can also slow tumor growth in animal models (**Vissers and Das, 2018**). This study aims to evaluate the exact cardiovascular effects of azithromycin in rats by focusing on histopathological changes and possible protective effects of VIT. C.

Materials and Methods

1. Drugs: Azithromycin and VIT.C were purchased from Nile Company for pharmaceuticals, Egypt.

2. Animals: Thirty male albino rats were used for the study, with an average weight of 170-250 gm. They were obtained from and housed in the Animal House of Sohag Faculty of Medicine, Egypt . Animal ethical considerations were completely fulfilled according to the guidelines of the Sohag University Committee for Animal Care and Use with approval certificate number 5-1-2022-1. They were kept under an environmental temperature of 23± 1°C. They were fed a standard pellet diet with free access to water. After acclimatization for one-week rats will be randomly divided into three groups.

3. Experimental Design: Rats were randomly divided into 3 equal groups as follows**:**

- **Group I (control group**); 10 rats received normal saline by oral gavage
- **Group II** (azithromycin group): They were treated with azithromycin in a dose of 30mg/kg orally daily for 2 weeks (**Atli et al., 2015**).
- **Group III** (azithromycin +VIT C): They were treated with azithromycin as the previous group +VIT C (20 mg/kg) orally daily for 2 weeks (**Swamy et al.,2011**).

24 hours after end of the experiment, rats were anesthetized, sacrificed, and dissected. Samples from the left ventricle of the heart were taken.

Histological studies

I . Light microscopic study:

- With Hematoxylin and Eosin (H&E)(**Bancroft & Gamble, 2002**).
- Masson trichrome stains (MT) (**Chen et al., 2017**).

II. Immunohistochemical staining: Sections were boiled in 10 mm citrate buffer (AP9003) at pH 6 for 10 minutes to retrieve antigen, then incubated for 1h with the following antibodies;

- Caspase-3 (rabbit polyclonal antibody, ab13847)for apoptosis (purchased from Abcam, MA, USA) (**Mohamed & Kassem, 2018**).
- Tumor necrosis factor- alpha (TNF-α)(stained with avidin-biotin peroxidase) (**Hora et al., 2005**).

Sections were stained with Mayer's hematoxylin stain (TA060-MH). A positive reaction appeared as brown discoloration. Citrate buffer, Ultra vision detection system, and Mayer's hematoxylin were purchased from Lab visionThermo Scientific, Fremont, California, USA. Antibodies against TNF-α were purchased from (Minneapolis, Minnesota, USA).

Morphometric and statistical study

-The following measures were taken:

- Area percent of collagen **(Schipke et al., 2017).**
- Area percent of caspase-3 immunoreaction was measured

using an objective lens of x40 magnification (**Mansour et al., 2021**).

10 non-overlapping fields for each section were taken. This was done using Image J software (version 1.51k, Wayne Rasband, National Institutes of Health, USA). From each variable, the mean± standard deviation of the meanwas measured using SPSS program version(16).a post-hoc test was used to find the statistical difference between the groups when ANOVA was statistically significant (P value ≤0.05) (**Eid et al., 2020**).

Results

Histopathological Results

A) Control group

Histological examination showed the appearance of cardiac muscle fibers with regular striations. The cardiac myocytes had normal architecture with acidophilic cytoplasm and central oval nuclei. They were arranged in bundles with narrow spaces in between **(Fig.1).** Using Masson trichomestain, showed minimal collagen fibers between the cardiac myocyte **(Fig.4).**

B) Azithromycin treated group

Examination of this group revealed loss of cardiac muscle striation. with distortion, and fragmentation of myocardial fibers. Cells of myocardial fibers showed vacuolated cytoplasm and peripheral pyknotic nuclei. Areas of intracellular bleeding also appeared **(Fig.2).** In the Masson trichome stain, there was a highly significant increase in the area % of collagen fibers (p≤0.002) **(Fig.5).**

C) Azithromycin and VIT. C treated group:

This group showed restoration of the near normal appearance of cardiac muscle fibers. Few myocytes represented degenerated nuclei with vacuolated cytoplasm also some other cells have small atrophic nuclei. Some congested dilated blood vessels and little cellular infiltration also appeared **(Fig.3).** In the Masson trichome stain of this treated group the collagen fibers decreased with non-significant change from the control(p=0.8) **(Fig.6).**

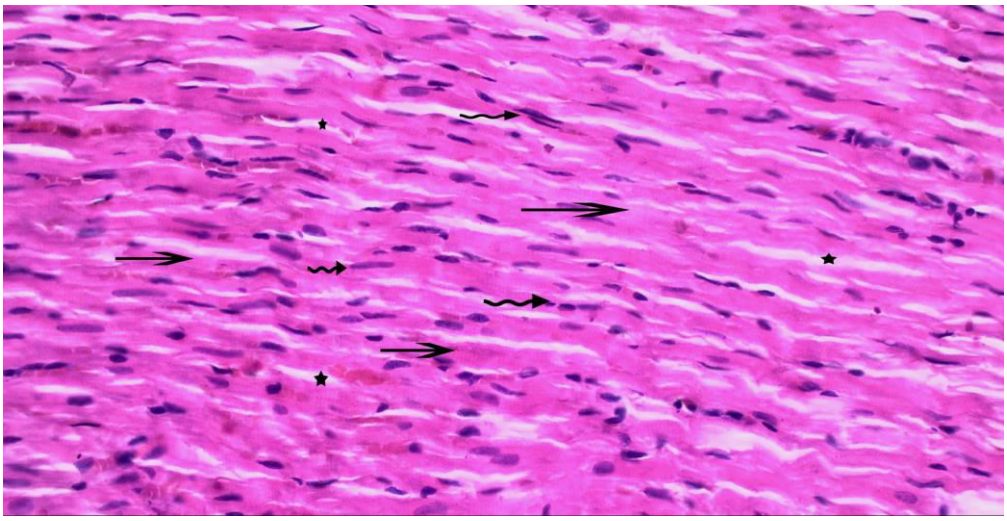

Fig.1. A photomicrograph of the left ventricle of a control rat showing a regular arrangement of cardiac muscle fibers with acidophilic cytoplasm **(arrows)** and central oval vesicular nuclei **(irregular arrow)** of cardiac myocytes. Myofibers are arranged in bundles with narrow spaces in between **(*) (H&E X400).**

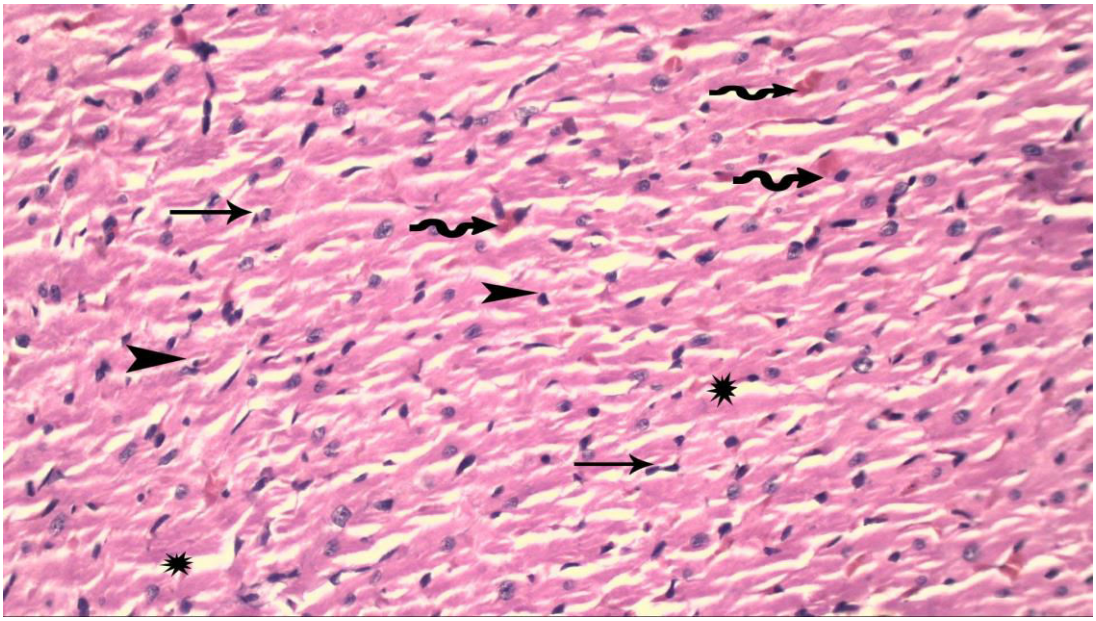

Fig.2. A photomicrographic picture of the left ventricle of an azithromycin-treated rat showing marked distortion, and fragmentation of cardiac muscle fiber where myofibers were distorted ( ), area of little bleeding appeared **(irregular arrow).** Signs of myocardial necrosis in the form of cytoplasmic vacuolation **(arrow)** and small atrophic pyknotic nuclei **(arrowhead). (H&E X400).**

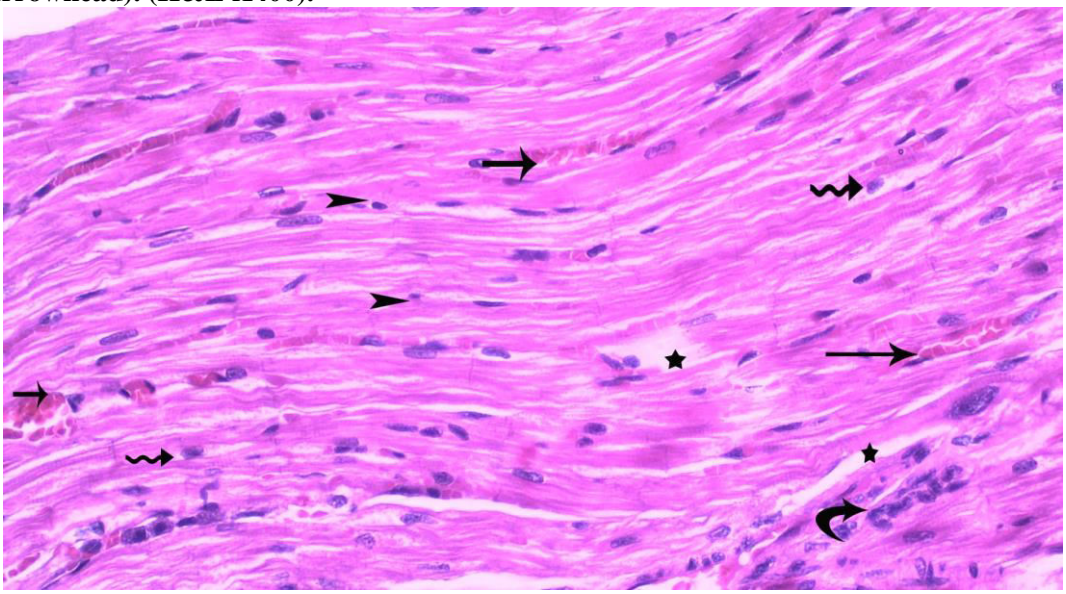

Fig.3. A photomicrographic picture of the left ventricle of azithromycin and VIT. C treated rat showing a restored arrangement of cardiac muscle fibers with narrow spaces in between**(stars).** Few myocytes represent degenerated nuclei with vacuolated cytoplasm**(irregular arrow)** some other cells have a small atrophic nucleus**(arrowhead).** Some congested dilated blood vessels **(arrows)** and little cellular infiltration were obs**erved (curved arrow). (H&E X400).**

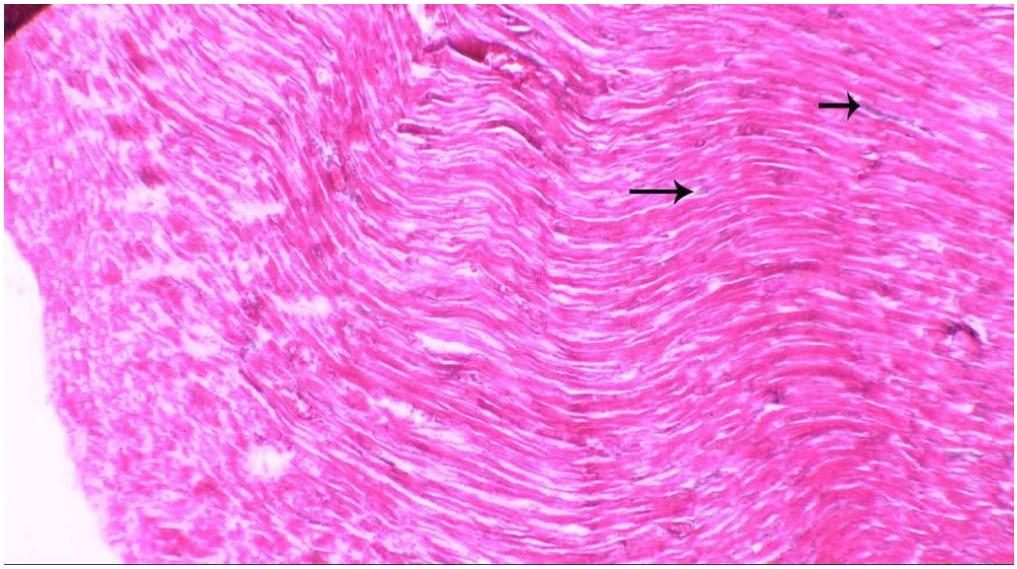

Fig.4. A photomicrograph of the left ventricle of a control rat showing scanty collagen fibers between fibers of cardiac muscles**(arrows). Masson trichrome×200**

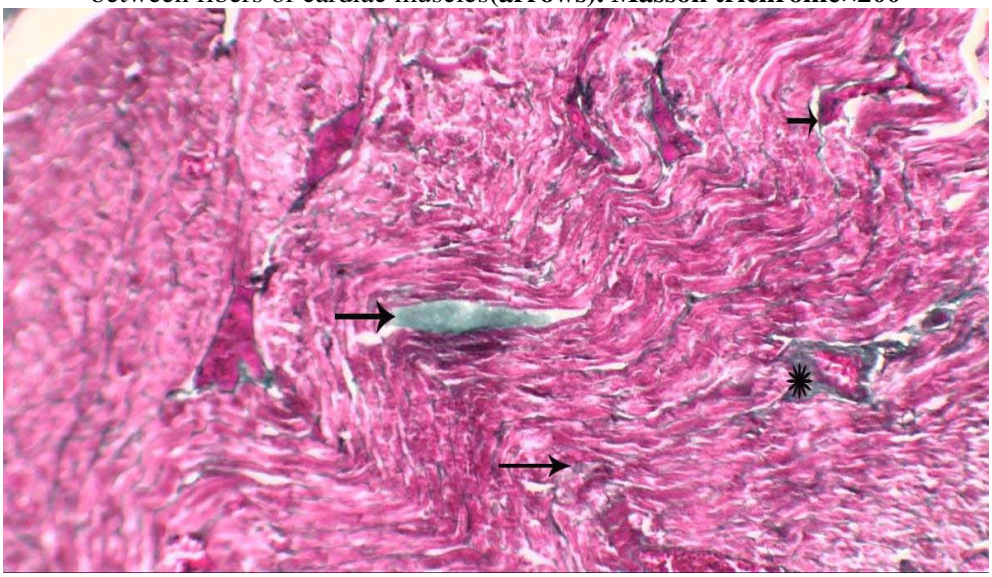

Fig.5. A photomicrograph of the left ventricle of rats treated with Azithromycin showed a marked increase in collagen fibers between the muscles **(arrows)** and congested blood vessels **(star).Masson trichrome ×200.**

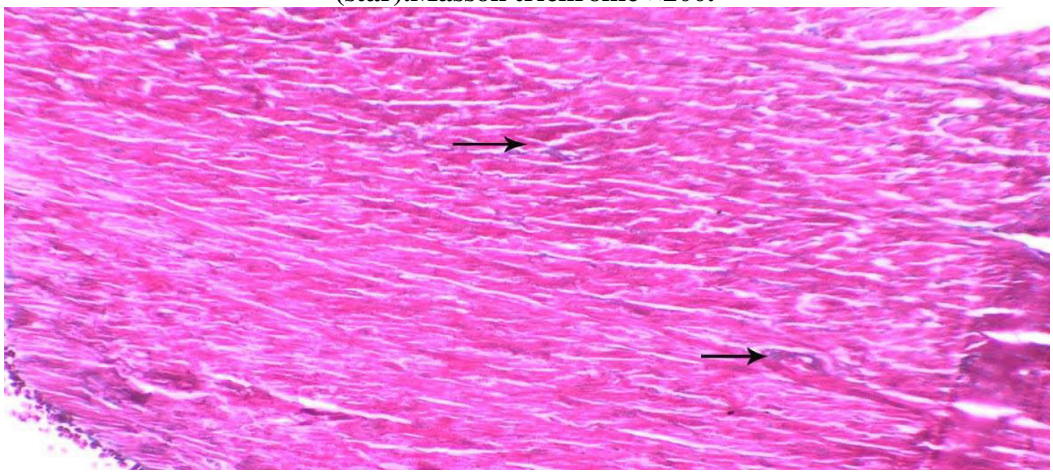

Fig.6. A photomicrograph of the left ventricle of rats treated with Azithromycin and VIT.C showed few collagen fibers between the muscles**(arrows).Masson trichrome×200**

Immunohistochemistry

A) Caspase-3: It appeared as a brownish coloration either cytoplasmic or nuclear. In the control group, minimal expression of caspase-3 was seen **(Fig.7),** in 2nd group the expression (brown stain) of caspase-3 highly significantly increased compared with the control (p≤ 004) **(Fig. 8)**, in VIT.C and azithromycin, treated group, brown stain decreased significantly (p≤0.045) from the previous group and became near normal (p=0.4) (moderate expression) **(Fig.9).**

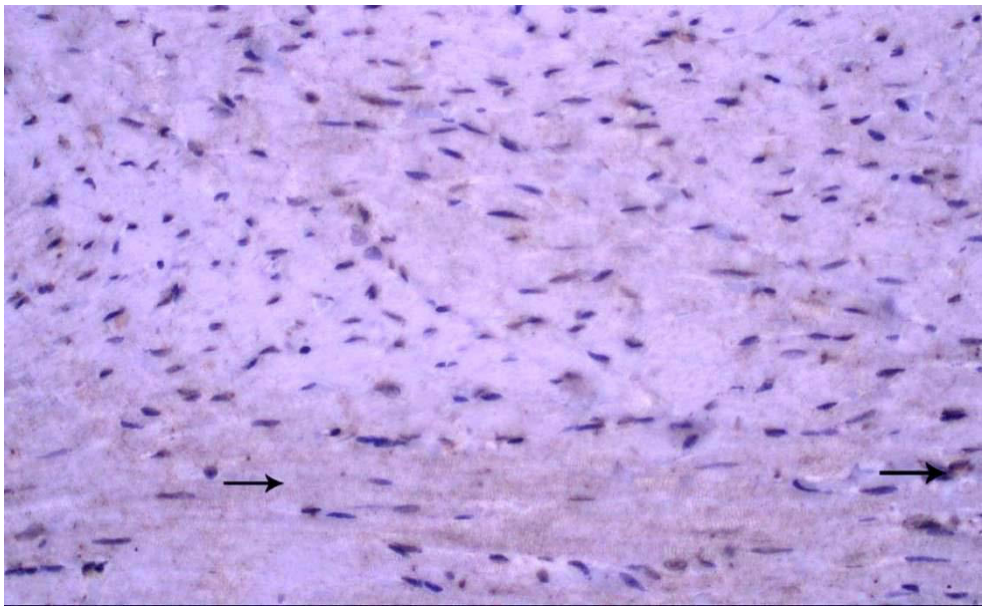

Fig.7.A photomicrograph of the left ventricle of a control rat showing minimal expression of caspase-3:brown stain**(arrows). (×400)**

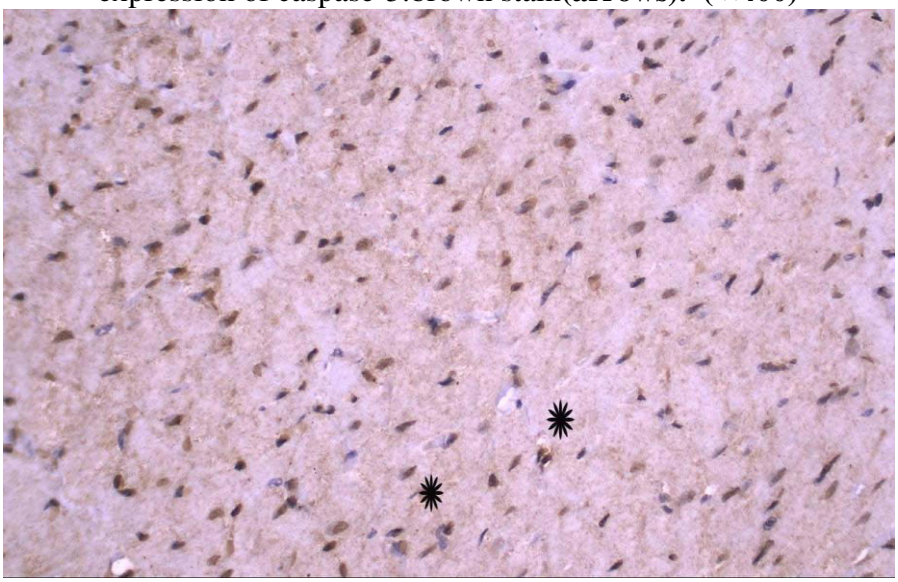

Fig.8. A photomicrograph of the left ventricle of rats treated with Azithromycin showed more expression of caspase -3reaction**(stars). (×400).**

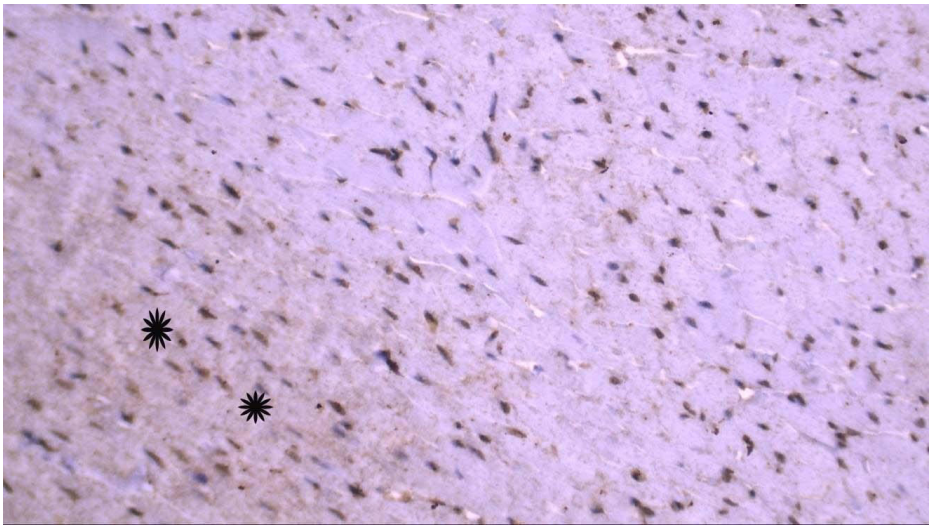

Fig.9. A photomicrograph of the left ventricle of rats treated with Azithromycin and VIT.C showed a weak caspase-3 reaction **(stars). (×400).**

B) TNF-α: In the control group negative reaction was seen **(Fig.10).**While in the azithromycin-treated group the reaction was positiveeither cytoplasmic or nuclear**(Fig. 11)** and in the VIT.C and azithromycin-treated group a week positive reaction was observed **(Fig.12).**

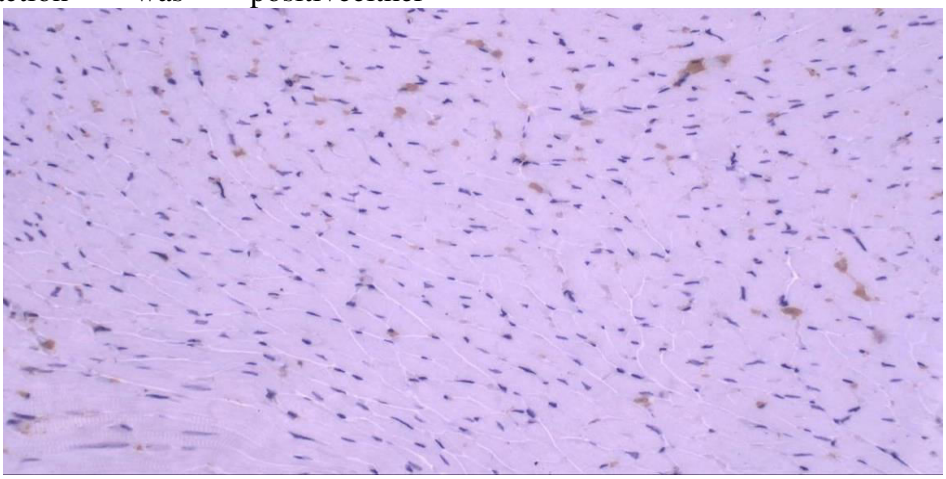

Fig.10. A photomicrograph of the left ventricle of control rats showing a negative reaction of TNF-α between the muscles**. (×400)**

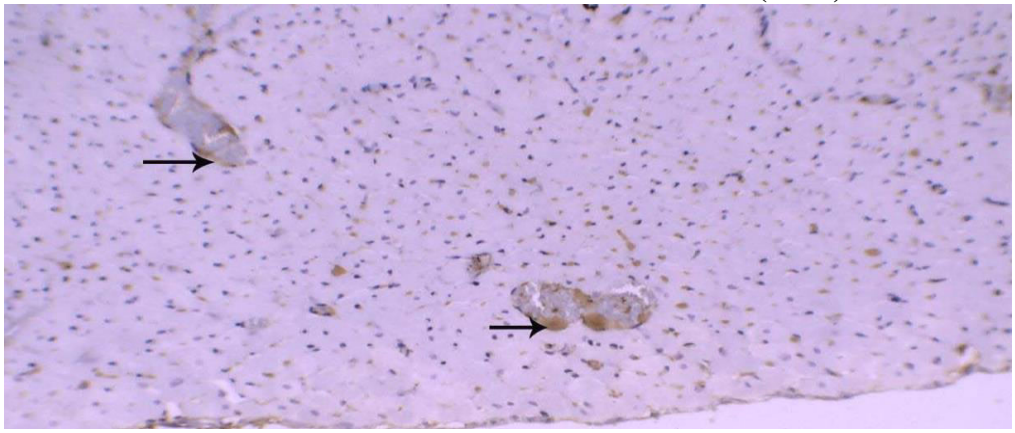

Fig.11. A photomicrograph of the left ventricle of rats treated with Azithromycin showed a positive reaction of TNF-α**(arrows). (×400)**

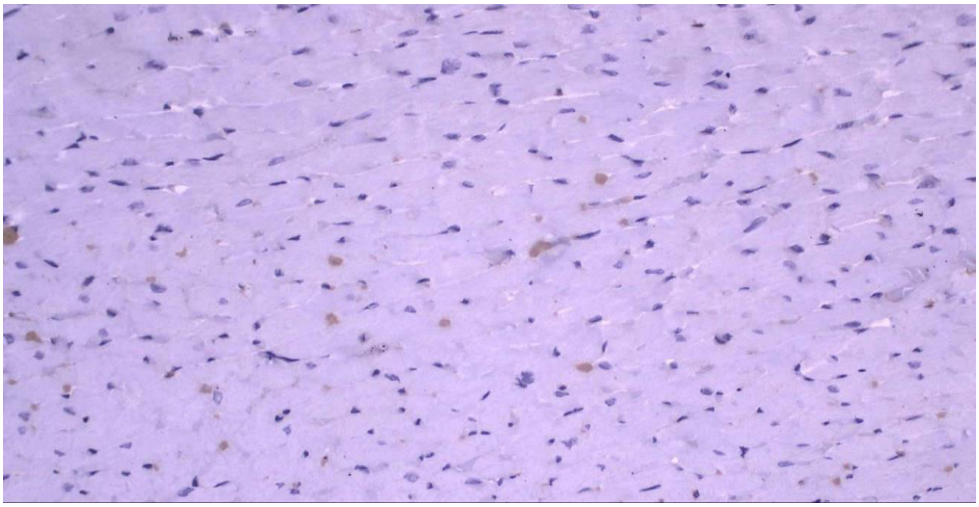

Fig.12. A photomicrograph of the left ventricle of rats treated with Azithromycin and VIT.C showed a negative reaction of TNF-α between the muscles. **(×400)**

Morphometric Results

A) Masson Area %: The mean area percent of Masson trichome stain in the control group was16.34±2.24, and the mean collagen area percent in the Azithromycin group was 29.47±2.22 with a highly significant increase in the control group($p \leq 0.002$), the 3rd group (VIT.C), there was a significant decrease in the mean collagen area percent (17.11±2.88) than the previous group($p \leq 0.02$), while a non-significant change from the control group ($p=0.8$) **(Table1, Fig.13).**

Table 1. Themean Area % of collagen fibers and caspase-3 expression in different groups

Variables	Control	Azithromycin	VIT.C
Masson Area%	16.34±2.24	29.47±2.22**	17.11±2.88
Caspase Area%	4.94 ±0.98	14.06±2.48**	6.76±1.62

P > 0.05 (NS) → No significant difference.
P ≤ 0.05 (*) → Significant difference.
P ≤ 0.0l (**) → High significant difference.
P ≤ 0.001 (***) → Very high significant difference.

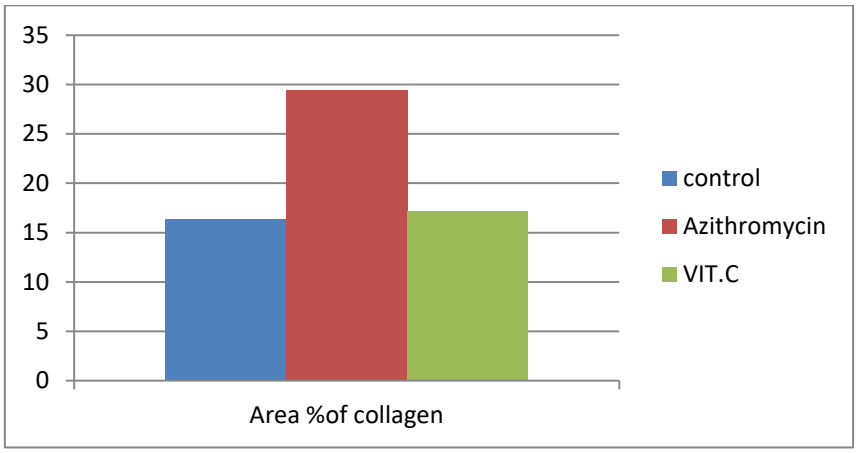

Fig.13. Mean Area% of collagen fibers in all groups

B) Caspase Area%: The mean area percent of caspase expression in the control group was 4.94±0.98, and the mean caspase expression percent in Azithromycin group was14.06±2.48, with a highly significant increase in the control group ($p \leq 0.004$), in 3rd group VIT.C group, the mean caspase expression percent was 6.76±1.62 with significant decrease than the 2nd group($p \leq 0.04$) but non-significant change than the control group($p=0.4$) **(Table1, Fig. 14).**

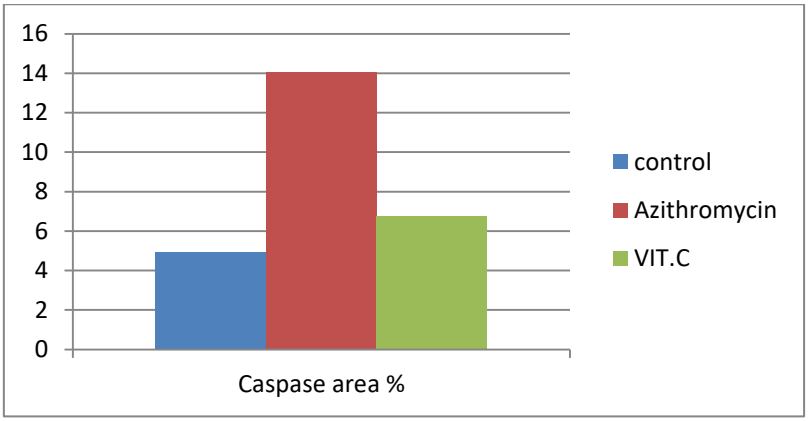

Fig.14. Mean Area % of caspase expression in all groups

Discussion

Erythromycin is a macrolide drug that is widely used as a broad-spectrum antibiotic **(Tsubouchi et al., 2017).** Azithromycin is a semi-synthetic 9-N-methylation derivative of erythromycin; it is recommended in the United States as a drug of choice in outpatient treatment or as part of combination therapy in patients who require hospitalization. Azithromycin is used as a first-line treatment for pneumonia for over 15 years **(Wang et al., 2018).**

The protective effect of azithromycin for the treatment of SARS and Covid pneumonia was approved by **Parnham et al. (2014) and Beigel et al. (2020)** due to its immunomodulatory activity. However, **Martín-Loeches et al. (2013)** approved the cardiotoxic effect of azithromycin in humans.

In the present study, rats treated with azithromycin showed histopathological changes in the form of loss of cardiac muscle striation and distortion of the myocardial fiber. Cells showed vacuolation of cytoplasm and peripheral pyknotic nuclei and the presence of an area of intracellular bleeding. With the Masson trichome stain, there was an increase in collagen reaction. This may be due to increased free-radical formation which induced oxidative damage to cellular lipids, proteins, and DNA (**Pacher et al., 2005).**These findings agreed with that of **Atli et al., (2015); El-Shitany and El-Desoky(2016); Mansour et al., (2021)** who showed that treatment of rats with azithromycin resulted in marked atrophy of cardiac muscle fibers with increased tissue spaces with dilated damaged arteries. This means that azithromycin-induced negative effects on cardiac muscle due to

disturbances in antioxidant/oxidant balance and increase apoptosis in the cells.

Using Masson's trichrome staining show different degree of cardiac fibrosis. This was in line with **Abd El-Kader (2019**) who reported more collagen fibers with the azithromycin group. This could be explained that increased free-radical formation induced more fibrosis in cardiac muscles.

In caspase-3, the azithromycin group showed increased reaction compared with the control group**..** This finding was accepted by **Agosto et al., (2011**); **El-Shitany& El-Desoky (2016)** who detected that treatment of rats with azithromycin resulted in marked caspase-3 expression.**)**

In previous studies; more expression of TNF-α appeared in the azithromycin group compared with the control group where AZ administration increased the oxidative stress and inflammatory response as signified by increased plasma TNF-α. (**Shin et al.,2002 ; Cai et al., 2013**).

In a cohort study of more than 7.8 million antibiotic exposures, there was a statistically significantly increased relative and absolute risk of cardiovascular death associated with azithromycin **(Jonathan et al., 2020).** On the other hand, some studies found that azithromycin is safe for the heart, **Andreaniet al. (2010) and Beigel et al. (2020)** found that its use has been related to a reduction of some factors such as IL-12, TNF-α, and GM-CSF. Other studies said that Azithromycin is anti-inflammatory to the heart and it can inhibit fibroblast proliferation, and collagen production. (**Al-Darraji et al., 2018; Deretic andTimmins, 2020**).

VIT.C isone of the natural cheap available nontoxic water-soluble antioxidant trapping radicals and protecting DNA, lipids, and proteins from oxidative damage **(Elsaid and Khattab, 2018).** In our study VIT. C diminished most of the hazards done by azithromycin and this was clear with light microscopic examination either with H and E stains or immunohistochemical markers.

Magdyet al. (2016) reported that VIT. C ameliorates organophosphate pesticide-induced damage in humans and animals. VIT.C protected rats against most azithromycin hazards by reducing elevated IL-1 and TNF-α levels and delayed the caspase-3 expression which means an anti-inflammatory and anti apoptotic cardioprotective effect against cardiotoxicity induced by azithromycin (**Abdel-Daim et al., 2015 ; Altuntas et al., 2004**).

Conclusion

Azithromycin is a cardiotoxic drug that should be used in limited cases. The toxic effects of azithromycin on the heart can be potentially reduced by the administration of VIT. C. Using VIT. C could be the best choice to avoid cardiac toxicity caused by azithromycin.

Conflict of interest:

No conflict of interest

Funds:

No funds

References

- **Abd El-kader M. (2019).** Evaluation of Azithromycin Induced Cardiotoxicity in Male

Albino Rats and the Possible Protective Role of Nigella Sativa Oil.Egyptian Journal of Histology,43(2).

- **Abdel-Daim MM , Ghazy EW, M. Fayez M. (2015).** "Synergistic protective role of miracle (Commiphoramolmol) and ascorbic acid against tilmicosin-induced cardiotoxicity in mice," Canadian Journal of Physiology and Pharmacology, 93 (1): 45–51.
- **Agosto M, Azrin M, Singh K, Jaffe AS, Liang BT. (2011).** Serum caspase-3 p17 fragment is elevated in patients with ST-segment elevation myocardial infarction: a novel observation. Journal of the American College of Cardiology, 57: 220-221.
- **Al-Darraji A, Haydar D, Chelvarajan L, Tripathi H, Levitan B, Gao E, et al. (2018) .** Azithromycin therapy reduces cardiac inflammation and mitigates adverse cardiac remodeling after myocardial infarction: Potential therapeutic targets in ischemic heart disease. PLoS ONE 13(7): e0200474.
- **Andreani J, Le Bideau M, Duflot I, et al. (2010).**In vitro testing of combined hydroxychloroquine and azithromycin on SARS-CoV-2 shows a synergistic effect. Microbial Pathogenesis, 145:104228.
- **Arrigoni O, De Tullio MC. (2002).** Ascorbic acid: Much more than just an antioxidant. Biochemical et Biophysical Acta. 1569: 1– 9.
- **Atli O, Ilgin OS, Altuntas H, Burukoglu D. (2015).** Evaluation of azithromycin induced cardiotoxicity in rats, International Journal of Clinical and Experimental Medicine; 8(3): 3681–3690.
- **Autifi MA, Mohamed WY, Abdul Haye WM, Elbaz KR. (2018).** The Possible Protective Role of VIT. C against Toxicity Induced by Lead Acetate in Liver and Spleen of Adult Albino Rats (Light and Electron Microscopic Study) The Egyptian Journal of Hospital Medicine 73 (10): 7650-7658.
- **Bancroft JD, Gamble M. Theory and practice of the histological techniques. In: Bancroft JD, Gamble M. (2002).** The Hematoxylin and Eosin, 5th end; Chp.8:125-139. Churchill Livingstone, London**. 17.**
- **Beigel JH, Tomashek KM, Dodd LE, et al. (2020).** Remdesivir for the treatment of Covid-19 — preliminary report. N Engl J Med, NEJMoa2007764. Available from:HTTP:// www.nejm. org/DOI/10.1056/NEJMoa2007764 10. 1056/ NEJMoa 2007764.
- **Budin SB, Han KJ, Jayusman PA, Taib IS, Ghagali AR, Mahamed J. (2011).** Antioxidant activity of tocotrienol-rich fraction prevents fenitrothion-induced renal damage in rats. Journal of Toxicologic Pathology, 26:111–118
- **Cai X, Lu W, Yang Y, Yang J, Ye J, and Gu Z. (2013).** Digitoflavone inhibits IκBα kinase

and enhances apoptosis induced by TNF- α through down-regulation of expression of nuclear factor κBregulated gene products in human pancreatic cancer cells, PLoS ONE, 8(10): ID 77126, 2013.

- **Chen Y, Yu Q, Xu1 C-B. A. (2017). a** convenient method for quantifying collagen fibers in atherosclerotic lesions by ImageJ software. Int J Clin Exp Med, 10(10):14904-14910.
- **Deretic V and Timmins GS. (2020).** Azithromycin and ciprofloxacin have a chloroquine-like effect on respiratory epithelial cells. bioRxiv.;p. 008631:Available from doi:10.1101/2020.03.29.008631.
- **Eid RA, Zaki MS, Alghamdi MA , Ali SA , Andarawi M &Haidara MA. (2020).** Vitamin CAdministration Attenuated Artemether- Induced Hepatic Injury in Rats. International Journal of Morphology, 38(1): 48-55.
- **Elsaid AG and Khattab RT. (2018).** The protective role of vitamin C against chlorine-induced lung injury in adult albino rats: Histological and immunohistochemical study, 40(4): 5515**.**
- **El-Shitany NA and El-Desoky K. (2016).** Protective Effects of Carvedilol and Vitamin C against azithromycin-Induced Cardiotoxicity in Rats via decreasing ROS, IL1-β, and TNF-α Production and Inhibiting NF-κB and Caspase-3 Expression, Oxidative Medicine and Cellular Longevity, Article ID, 1874762, 13 pages.
- **Ergul Y, Erkan T, Uzun H, Genc H, Altug T and Erginoz E. (2010) .**Effect of vitamin C on oxidative liver injury due to isoniazid in rats. Pediatrics International 52: 69–74.
- **Fohner AE, Sparreboom A, Altman RB, Klein TE. (2017).** pharmgkb summary: Macrolide antibiotic pathway, pharmacokinetics /pharmacodynamics. Pharmacogenet Genomics, 27(4):164-167.
- **Hemilä H(2017).** Vitamin C and Infections, Nutrients, 9(4): 339.
- **Hora K, Valença SS, Cristovao Porto L. (2005).** Immunohistochemical study of tumor necrosis factor-α, matrix metalloproteinase-12, and tissue inhibitor of metalloproteinase-2 on alveolar macrophages of BALB/c mice exposed to short-term cigarette smokeExperimental Lung Research. 31:759–770.
- **Kanoh S and, Rubin BK. (2010).** Mechanisms of action and clinical application of macrolides as immunomodulatory medications. Clinical Microbiology Reviews, 23(3): 590–615.
- **Li, Y. F, Ouyang, S. H, Tu, L. F, Wang, X, Yuan, W. L, Wang, G. E, Wu, Y. P, Duan, W. J, Yu, H. M, Fang, Z. Z, et al. (2018).** Caffeine protects the skin from oxidative stress-induced senescence through the activation of autophagy. Theranostics, 8(20): 5713-30, 2018.

- **Magdy W, Mohamed EF, Amin AS, Sarhan Rana SS. (2016).** Ameliorative effect of antioxidants (vitamins C and E) against abamectin toxicity in liver, kidney, and testis of male albino rats. The Journal of Basic & Applied Zoology,77: 69-82
- **Mansour BS, Salem NA, Abdel Kader G, Abdel-Alrahman G, Mahmoud OM. (2021).** Protective effect of Rosuvastatin on Azithromycin induced cardiotoxicity in a rat model Life Sciences, 15, 269: 119099.
- **Martín-Loeches I, Bermejo-Martin JF, Vallés J, et al. (2013).** Macrolide-based regimens in absence of bacterial co-infection in critically ill H1N1 patients with primary viral pneumonia. Intensive Care Med, 39 (4): 693–702**.**
- **Mc Gregor G and Biesalski H. (2006).** Rationale and impact of vitamin C in clinical nutrition. Curr. Current Opinion in Clinical Nutrition & Metabolic Care, 9: 697–703
- **Mmullan BJ, Mostaghim M. (2015):** Prescribing azithromycin. Aust Prescr, 38(3): 87-9.
- **Mohamed EA and Kassem HH. (2018).** Protective effect of nebivolol on doxorubicin-induced cardiotoxicity in rats. Archives of Medical Science, 14(6):1450-1458.
- **Pacher P, Schulz R, Liaudet L, Szab´o C. (2005).** Nitrosative stress and pharmacological modulation of heart failure. Trends in Pharmacological Sciences, 26(6): 302–310
- **Padhani ZA, Moazzam Z, Ashraf A, Bilal H, Salam RA, Das JK, Bhutta ZA. (2021).** Vitamin C supplementation for prevention and treatment of pneumonia. Cochrane Database Syst Rev, Nov 18;11(11): CD013134.
- **Parnham MJ, Erakovic-Haber V, Giamarellos-Bourboulis EJ, Perletti G, Verleden GM, Vos R. (2014) .** Azithromycin mechanisms of action and their relevance for clinical applications. Pharmacology & Therapeutics is a medical journal, 143: 225-245.
- **Parnham MJ, Haber VE, Giamarellos-Bourboulis EJ, et al. (2014).** Azithromycin: mechanisms of action and their relevance for clinical applications. journal of Pharmacology and Experimental Therapeutics, 143(2): 225–245.
- **Ray WA, Murray KT, Hall K, Arbogast PG, Stein CM. (2013).** Azithromycin and the risk of cardiovascular death. The New England Journal of Medicine, 366: 1881-1890.
- **Schipke J, Brandenberger C, Rajces A, Manninger M, Alogna A, Post H et al. (2017).** Assessment of cardiac fibrosis: a morphometric method comparison for collagen quantification. Journal of Applied Physiology, 122(4):1019-1030
- **Shin WS, Szuba A and Rockson SG. (2002).** The role of chemokines in human cardiovascular pathology: enhanced biological insights, Atherosclerosis, 160(1): 91–102.

- **Shireen KF, Pace RD, Mahboob M, and Khan AT. (2008).** Effects of dietary vitamin E, C, and soybean oil supplementation on antioxidant enzymes activated in liver and muscles of rats. Food. chemical toxicology journal, 46, 3290–3294.
- **Swamy AH, Wangikar U, Koti BC, Thippeswamy AH, Ronad PM, and Manjula DV. (2011).** Cardioprotective effect of ascorbic acid on doxorubicin-induced myocardial toxicity in rats, Indian Journal of Pharmacology, 43(5): 507-511.
- **Tsubouchi K, Araya J, Minagawa S, Hara H, Ichikawa A, Saito N, et al. (2017).** Azithromycin attenuates myofibroblast differentiation and lung fibrosis development through proteasomal degradation of NOX4, Autophagy 13: 1420-1434.
- **Vissers MC and Das AB.(2018).**Potential Mechanisms of Action for Vitamin C in Cancer. Reviewing the Evidence. Frontiers in Physiology. 9 (809).
- **Wang Q, Mi G, Hickey D, Li Y, Tu J, Webster TJ, et al. (2018).** Azithromycin-loaded respirable microparticles for targeted pulmonary delivery for the treatment of pneumonia, Biomaterials, 160:107–123.
- **Zaroff JG, Cheetham TC, Niki Palmetto P, Almers L, Quesenberry C, Schneider J, Gatto N, Corley D. (2020).** Association of Azithromycin Use With Cardiovascular Mortality. The Journal of the American Medical Association Network Open, 3(6): e208199. doi:10.1001/ JAMA network open. 8199.